## **EAST Search History**

| Ref<br># | Hits | Search Query  | DBs                | Default<br>Operator | Plurals | Time Stamp       |
|----------|------|---|--------------------|---------------------|---------|------------------|
| L1       | 3850 | (collagen or procollagen) near5<br>(recombinant\$ or express\$) | US-PGPUB;<br>USPAT | ADJ                 | OFF     | 2007/11/02 15:08 |
| L2       | 4004 | propeptide\$  | US-PGPUB;<br>USPAT | ADJ                 | OFF     | 2007/11/02 15:08 |
| (L3)     | 73   | 1 same 2  | US-PGPUB;<br>USPAT | ADJ                 | OFF     | 2007/11/02 15:09 |

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* * STN
                                 Columbus
FILE 'HOME' ENTERED AT 17:27:28 ON 02 NOV 2007
=> fil .bec
COST IN U.S. DOLLARS
                                                   SINCE FILE
                                                                   TOTAL
                                                        ENTRY
                                                                 SESSION
FULL ESTIMATED COST
                                                         0.21
                                                                    0.21
FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 17:27:40 ON 02 NOV 2007
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.
11 FILES IN THE FILE LIST
=> s (collagen or procollagen) (15a) (recombinant? or express?)
FILE 'MEDLINE'
        116079 COLLAGEN
          7982 PROCOLLAGEN
        290054 RECOMBINANT?
       1138855 EXPRESS?
         10382 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
L1
FILE 'SCISEARCH'
         98879 COLLAGEN
          6907 PROCOLLAGEN
        173117 RECOMBINANT?
       1459724 EXPRESS?
L2
         10606 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
FILE 'LIFESCI'
         16416 COLLAGEN
          1235 PROCOLLAGEN
         79059 RECOMBINANT?
        469304 EXPRESS?
L3
          2882 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
FILE 'BIOTECHDS'
          3459 COLLAGEN
           133 PROCOLLAGEN
        109482 RECOMBINANT?
        161686 EXPRESS?
           692 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
L4
FILE 'BIOSIS'
        118709 COLLAGEN
          6613 PROCOLLAGEN
        213599 RECOMBINANT?
       1386491 EXPRESS?
L_5
         12079 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
FILE 'EMBASE'
         97223 COLLAGEN
          7456 PROCOLLAGEN
        193336 RECOMBINANT?
       1049905 EXPRESS?
1.6
          9598 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
FILE 'HCAPLUS'
         94255 COLLAGEN
          5435 PROCOLLAGEN
        211433 RECOMBINANT?
       1383084 EXPRESS? ·
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11045 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)

L7

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791 COLLAGEN
            12 PROCOLLAGEN
          1888 RECOMBINANT?
         41482 EXPRESS?
             31 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
L8
FILE 'ESBIOBASE'
         28196 COLLAGEN
          1925 PROCOLLAGEN
         97679 RECOMBINANT?
        668120 EXPRESS?
          6252 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
L9
FILE 'BIOTECHNO'
         19647 COLLAGEN
          2536 PROCOLLAGEN
        127206 RECOMBINANT?
        452182 EXPRESS?
          4310 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
L10
FILE 'WPIDS'
         17287 COLLAGEN
           219 PROCOLLAGEN
         52890 RECOMBINANT?
        152537 EXPRESS?
           629 (COLLAGEN OR PROCOLLAGEN) (15A) (RECOMBINANT? OR EXPRESS?)
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TOTAL FOR ALL FILES
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=> s 112 and propeptide#
FILE 'MEDLINE'
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L13
           147 L1 AND PROPERTIDE#
FILE 'SCISEARCH'
          4268 PROPEPTIDE#
           199 L2 AND PROPEPTIDE#
L14
FILE 'LIFESCI'
          1183 PROPERTIDE#
L15
            50 L3 AND PROPEPTIDE#
FILE 'BIOTECHDS'
           278 PROPEPTIDE#
            18 L4 AND PROPERTIDE#
L16
FILE 'BIOSIS'
          4094 PROPEPTIDE#
           148 L5 AND PROPERTIDE#
L17
FILE 'EMBASE'
          3402 PROPEPTIDE#
           138 L6 AND PROPEPTIDE#
L18
FILE 'HCAPLUS'
          3919 PROPERTIDE#
L19
           168 L7 AND PROPERTIDE#
FILE 'NTIS'
            14 PROPEPTIDE#
             0 L8 AND PROPEPTIDE#
L20
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FILE 'NTIS'

FILE 'ESBIOBASE'

2030 PROPEPTIDE#

L21 103 L9 AND PROPERTIDE#

FILE 'BIOTECHNO'

1648 PROPEPTIDE#

L22 95 L10 AND PROPERTIDE#

FILE 'WPIDS'

243 PROPEPTIDE#

L23 12 L11 AND PROPERTIDE#

TOTAL FOR ALL FILES

L24 1078 L12 AND PROPEPTIDE#

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6788357 1995-2007/PY

(19950000-20079999/PY)

L25 33 L13 NOT 1995-2007/PY

FILE 'SCISEARCH'

13244375 1995-2007/PY

(19950000-20079999/PY)

L26 32 L14 NOT 1995-2007/PY

FILE 'LIFESCI'

1554705 1995-2007/PY

L27 15 L15 NOT 1995-2007/PY

FILE 'BIOTECHDS'

255675 1995-2007/PY

L28 1 L16 NOT 1995-2007/PY

FILE 'BIOSIS'

7191596 1995-2007/PY

L29 33 L17 NOT 1995-2007/PY

FILE 'EMBASE'

6070648 1995-2007/PY

L30 31 L18 NOT 1995-2007/PY

FILE 'HCAPLUS'

12577537 1995-2007/PY

L31 31 L19 NOT 1995-2007/PY

FILE 'NTIS'

314593 1995-2007/PY

L32 0 L20 NOT 1995-2007/PY

FILE 'ESBIOBASE'

3607034 1995-2007/PY

L33 3 L21 NOT 1995-2007/PY

FILE 'BIOTECHNO'

1033893 1995-2007/PY

L34 27 L22 NOT 1995-2007/PY

FILE 'WPIDS'

9373698 1995-2007/PY

L35 0 L23 NOT 1995-2007/PY

TOTAL FOR ALL FILES

L36 206 L24 NOT 1995-2007/PY

=> dup rem 136

## => d tot

L37

- L37 ANSWER 1 OF 61 MEDLINE on STN DUPLICATE 1
- TI Interferon-alpha 2a increases serum concentration of hyaluronic acid and type III procollagen aminoterminal propeptide in patients with chronic hepatitis B virus infection.
- SO Digestive diseases and sciences, (1994 Sep) Vol. 39, No. 9, pp. 2007-13. Journal code: 7902782. ISSN: 0163-2116.
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- AN 94364144 MEDLINE
- L37 ANSWER 2 OF 61 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
- TI Altered Collagen Expression in Human Dentin: Increased Reactivity of Type III and Presence of Type VI in Dentogenesis Imperfecta, as Revealed by Immunoelectron Microscopy.
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- AN 1995:60782 BIOSIS
- L37 ANSWER 3 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
- TI EXPRESSION OF TYPE-II COLLAGEN AT THE MIDDLE STAGES OF CHICK EMBRYONIC AND HUMAN PETAL SKIN DEVELOPMENT
- SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (JUN 1994) Vol. 102, No. 6, pp. 958-962.
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- L37 ANSWER 4 OF 61 MEDLINE on STN DUPLICATE 2
- TI Effects of ascorbic acid on collagen matrix formation and osteoblast differentiation in murine MC3T3-E1 cells.
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- TI In situ expression of collagen and proteoglycan genes in notochord and during skeletal development and growth.
- SO Microscopy research and technique, (1994 Aug 15) Vol. 28, No. 6, pp. 470-82.
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- L37 ANSWER 7 OF 61 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
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- L37 ANSWER 9 OF 61 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5
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- AN 94264348 MEDLINE
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- AN 93206917 MEDLINE
- L37 ANSWER 25 OF 61 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
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- L37 ANSWER 27 OF 61 HCAPLUS COPYRIGHT 2007 ACS on STN
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- L37 ANSWER 28 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
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- AN 1992:195178 SCISEARCH
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- L37 ANSWER 30 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
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- AN 92011898 MEDLINE
- L37 ANSWER 38 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
- TI THE MOUSE COL2A-1 GENE IS HIGHLY CONSERVED AND IS LINKED TO INT-1 ON CHROMOSOME-15
- SO MAMMALIAN GENOME, (1991) Vol. 1, No. 3, pp. 171-183. ISSN: 0938-8990.
- AU CHEAH K S E (Reprint); AU P K C; LAU E T; LITTLE P F R; STUBBS L
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- L37 ANSWER 39 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
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- SO BIOCHEMICAL JOURNAL, (1 OCT 1991) Vol. 279, Part 1, pp. 49-54. ISSN: 0264-6021.
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- AN 1991:567047 SCISEARCH
- L37 ANSWER 40 OF 61 HCAPLUS COPYRIGHT 2007 ACS on STN
- TI Gene expression of type I, II and X collagens and osteonectin mRNAs in chick growth cartilage and cultured chondrocytes
- SO Shika Kiso Igakkai Zasshi (1991), 33(1), 1-15 CODEN: SHKKAN; ISSN: 0385-0137
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- AN 1991:599974 HCAPLUS
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- L37 ANSWER 11 OF 61 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

Apparently because the biosynthetic pathways involve eight or more AB highly specific post-translational enzymes, it has been difficult to obtain expression of genes for fibrillar collagens in recombinant systems. Here two constructs of the human gene for procollagen II (COL2A1) were prepared, one with about 0.5 kb of a promoter for a procollagen I gene (COL1A1) and the other with about 4 kb of the promoter for the procollagen II gene. The constructs, together with a neomycin-resistant gene, were transfected into a human tumour cell line (HT1080) that synthesizes the collagen IV found in basement membranes, but does not synthesize any fibrillar collagen. About two per 100 clones resistant to the neomycin analogue G418 synthesized and secreted human procollagen II. Milligram quantities of the recombinant procollagen II were readily isolated from the cultured medium. The recombinant procollagen II had the expected amino acid sequence as defined by nucleotide sequencing of mRNA-derived cDNA and the expected amino acid composition as defined by analysis of procollagen II that was converted into collagen II by digestion with procollagen N- and C-proteinases. Also, analysis of the carbohydrate content indicated that there was glycosylation of some of the hydroxylysine residues but no evidence of post-translational overmodification of the residues. In addition, the protein was shown to have a native conformation as assayed by a series of protease digestions. No essential differences were found between clones transfected with the COL2A1 gene construct containing the COL1A1 promoter and the similar construct containing the COL2A1 promoter in terms of number of clones synthesizing recombinant procollagen II and the levels of expression. With both constructs, the expression of the COL2A1 gene was closely related to copy number. results demonstrated therefore that it is not essential to use a promoter for a gene normally expressed in a host cell in order to obtain gene copy-number-dependent expression of an exogenous

MEDLINE on STN ANSWER 22 OF 61 DUPLICATE 12 Type II procollagen mRNAs are alternatively spliced: type IIA mRNA ΑB contains an exon encoding a cysteine-rich domain in the aminopropeptide and type IIB mRNA lacks this exon. In mouse embryos between 9.5 and 13.5 days, type IIA mRNA was the major form of Col2a-1 transcript expressed in both prechondrogenic and nonchondrogenic tissues and type IIB mRNAs were present in small amounts. After 12.5 days, type IIB mRNA levels increased rapidly and finally exceeded type IIA mRNAs. Type IIB mRNAs became the major Col2a-1 transcript by 14.5 days, predominantly expressed in maturing chondrocytes. By 17.5 days type IIB mRNAs account for 80% of the Col2a-1 transcripts. Expression of type IIA mRNAs follows the change in the growth pattern of the cartilaginous model of the axial and appendicular skeleton and of the otic capsule and nasal septum. In nonchondrogenic tissues, type IIA mRNAs are more commonly expressed in epithelial structures of ectodermal and endodermal origin than in nonepithelial tissues. The switching of expression from type IIA to type IIB mRNA as major Col2a-1 transcript may be associated with the commitment of precursor cells to the chondrocyte lineage and sites of type IIA mRNA expression may mark regions of potential cartilage growth. The differential expression pattern of type IIA mRNAs therefore points to an association of type IIA procollagen with chondrocyte differentiation during cartilage growth and some function early in embryogenesis in the epithelial organization of nonchondrogenic tissues.

collagen gene in stably transfected cells.

ANSWER 27 OF 61 HCAPLUS COPYRIGHT 2007 ACS on STN

AB A review, with 24 refs., of the authors' in vitro cell culture studies on the production of the lethal perinatal OI phenotype by the production of COL1A1 glycine mutations, the OI type I phenotype by the generation of a frameshift mutation, and an examination of the biochem. function of the proα1(I) propeptide high-mannose oligosaccharide by alteration of Asn-Ile-Thr attachment motif.

- DUPLICATE 15 ANSWER 32 OF 61 MEDLINE on STN L37 We have isolated overlapping cDNA clones from human and hamster libraries which comprise the entire coding sequences for the prepro-alpha 1(V) collagen chains of both species. The translated polypeptide has a signal peptide of 36 amino acids, a central triple helical domain of 338 uninterrupted Gly-X-Y triplets, and 266 amino acids which comprise the C-telopeptide and propeptide. The N-propeptide and telopeptide are comprised of 522 residues in humans and 524 residues in hamsters. The cDNA-derived pro-alpha 1(V) amino acid sequences exhibit a variety of structural features characteristic of fibrillar collagens. Pro-alpha 1(V) is found to be unique among fibrillar collagen chains, however, in lacking potential cross-linking lysyl residues in either telopeptide, and in possessing potential N-asparaginyl-linked carbohydrate attachment sites in its N-propeptide. Of particular interest is the strong homology found between the pro-alpha 1(V) and pro-alpha 1(XI) collagen chains in most domains, with the notable exception of a subdomain in the globular region of the N-propeptide. RNase protection analysis of RNA with a variety of pro-alpha 1(V) cDNA-derived riboprobes indicates a broad distribution of expression of the pro-alpha 1(V) chain in tissues and suggests that transcripts encoding the pro-alpha 1(V) chain and the putative pro-alpha 1'(V) chain are not products of the same gene.
- ANSWER 35 OF 61 HCAPLUS COPYRIGHT 2007 ACS on STN AΒ It was demonstrated previously that the C- and N-terminal propeptides of type I procollagen can inhibit procollagen synthesis by specifically decreasing procollagen mRNA levels. objective of the present expts. was to determine the mechanism by which propeptides cause these pretranslational effects. IMR-90 fibroblasts were exposed to medium containing C-terminal propeptide of type I procollagen, and nuclear run-off assays were performed by hybridization to a specific α1 chain type I procollagen cDNA probe. Specific type I procollagen transcription rates were decreased by 50% in the presence of 75 nM C-terminal propeptide compared with control (untreated) cells. Total cellular transcription rates as well as  $\beta$ -actin mRNA rates were not affected significantly by any concentration of C-terminal propeptide. Propeptide radiolabeled with 125I was taken up by cultured cells. Furthermore, exogenous C-terminal propeptide levels increased in the cytosolic compartment and eventually reached a steady-state level of 18 pmol/g cell protein by 30 Of particular interest was the finding that levels of radiolabeled C-terminal propeptide were also detected in the nuclear fraction and increased with time, reaching a plateau after 60 min of incubation. Incubation of nuclei from IMR-90 cells in medium containing varying concns. of C-terminal propeptide resulted in nuclear transcription rates that were decreased by 40% compared with untreated controls. nuclear message levels remained unchanged under identical conditions. Thus, the C-terminal propeptide of type I procollagen can be internalized and become associated with the nuclear compartment. suggests a feedback regulatory role on procollagen synthesis by a direct effect on procollagen gene transcription.
- DUPLICATE 18 ANSWER 36 OF 61 MEDLINE on STN L37 Type II collagen is a major component of cartilage providing structural AΒ integrity to the tissue. Type II procollagen can be expressed in two forms by differential splicing of the primary gene transcript. The two mRNAs either include (type IIA) or exclude (type IIB) an exon (exon 2) encoding the major portion of the amino (NH2)propeptide (Ryan, M. C., and L. J. Sandell. 1990. J. Biol. Chemical 265:10334-10339). The expression of the two procollagens was examined in order to establish a potential functional significance for the two type II procollagen mRNAs. First, to establish whether the two mRNAs are functional, we showed that both mRNAs can be translated and the proteins secreted into the extracellular environment. Both proteins were identified as type II procollagens. Secondly, to test the hypothesis that differential expression of type II procollagens may be a marker

for a distinct population of cells, specific procollagen mRNAs were localized in tissue by in situ hybridization to oligonucleotides spanning the exon junctions. Embryonic vertebral column was chosen as a source of tissue undergoing rapid chondrogenesis, allowing the examination of a variety of cell types related to cartilage. In this issue, each procollagen mRNA had a distinct tissue distribution during chondrogenesis with type IIB expressed in chondrocytes and type IIA expressed in cells surrounding cartilage in prechondrocytes. morphology of the cells expressing the two collagen types was distinct: the cells expressing type IIA are narrow, elongated, and "fibroblastic" in appearance while the cells expressing type IIB are large and round. The expression of type IIB appears to be correlated with abundant synthesis and accumulation of cartilagenous extracellular matrix. The expression of type IIB is spatially correlated with the high level expression of the cartilage proteoglycan, aggrecan, establishing type IIB procollagen and aggrecan as markers for the chondrocyte phenotype. Transcripts of type II collagen, primarily type IIA, are also expressed in embryonic spinal ganglion. While small amounts of type II collagen have been previously detected in noncartilagenous tissues, the detection of this new form of the collagen in relatively high abundance in embryonic nerve tissue is unique. Taken together, these findings imply a potential functional difference between type IIA and type IIB procollagens and indicate that the removal of exon 2 from the pre-mRNA, and consequently the NH2-propeptide from the collagen molecule, may be an important step in chondrogenesis. In addition, type II procollagen, specifically type IIA, may function in noncartilage tissues, particularly during development.

- ANSWER 41 OF 61 MEDLINE on STN **DUPLICATE 20** 1,37 AB Type II collagen, like other fibrillar collagens, is synthesized as a procollagen containing amino (NH2)-and carboxyl (COOH)-terminal extension peptides. Based on cDNA cloning of human (Baldwin, C. T., Reginato, A. M., Smith, C., Jimenez, S. A., and Prockop, D. J. (1989) Biochem. J. 262, 521-528) and rat (Kohno, K., Martin, G. R., and Yamada, Y. (1984) J. Biol. Chemical 259, 13668-13673) type II procollagen, it was concluded that much of the NH2-terminal propeptide seen in pro-alpha 1(I) was missing. Analysis of human genomic clones for type II collagen revealed an additional exon encoding a 69-amino acid cysteine-rich domain in the NH2-terminal propeptide. This exon (exon 2) is expressed in the mRNA population of chondrocytes isolated from human fetal skeleton and notochord, juvenile costal cartilage, and bovine articular cartilage. Oligonucleotide probes spanning specific exon boundaries were used to detect two populations of procollagen mRNA by Northern blot analysis. Amplification of cDNA templates using polymerase chain reaction provided direct evidence for two distinct pro-alpha 1(II) collagen mRNAs. DNA sequence analysis showed that the two mRNAs resulted from the alternative splicing of exon 2. The protein domain encoded by exon 2 is conserved between the fibrillar collagens and two other extracellular matrix proteins, thrombospondin and von Willebrand factor. In fibrillar collagens, this protein domain may play a regulatory role in fibrillogenesis and feedback inhibition of collagen biosynthesis. Consequently, the differential expression of this protein domain could alter the biosynthesis or fibril formation of type II collagen. In addition, the expression of exon 2 may be a marker for a distinct population of chondrocytes.
- ANSWER 42 OF 61 MEDLINE on STN DUPLICATE 21

  AB We have determined the nucleotide sequence of several overlapping cDNA clones encoding the amino-terminal portion of human alpha 1(XI) procollagen. These experiments have revealed that this domain of the pro-alpha(XI) chain displays structural features common to other fibrillar procollagen molecules, such as a putative amino-terminal proteinase cleavage site and an interrupted collagenous segment. In the latter, structural similarities were noted when alpha 1(XI) was compared with

alpha 1(II) and alpha 2(V) procollagens. Overall, however, the amino-terminal region of pro-alpha 1(XI) differs greatly in composition and size from that of other fibrillar chains. Nearly three-fourths of this domain is in fact composed of a 383-amino acid globular region in which a 3-cysteine cluster signals the transition to a long and highly acidic carboxyl-terminal segment. Finally, the unrestricted expression of this cartilage-specific collagen gene has been confirmed by the finding of high levels of pro-alpha 1(XI) mRNA in two human rhabdomyosarcoma cell lines.

- L37 ANSWER 43 OF 61 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
- **DUPLICATE 22** MEDLINE on STN L37 ANSWER 44 OF 61 Peptides corresponding to selected sequences of the alpha 1 chain of the COOH propeptide of type I and type III human procollagen were synthesized and used as antigens to develop polyclonal and monoclonal antibodies. The antibodies were shown to be epitope specific using a peptide-based solid phase enzyme-linked immunoadsorbent assay. antibodies were specific for the appropriate procollagens and the COOH propeptides isolated from serum-free culture supernatants of human skin fibroblasts. The rabbit antisera directed to the type I synthetic peptide bound the intact procollagen molecule and both the procollagen alpha 1(I) and alpha 2(I) chains after the reduction of the disulfide In addition, the antisera bound intact type I COOH propeptide, generated by bacterial collagenase treatment of procollagen, and the individual chains of the propeptide after reduction. In contrast, a monoclonal antibody to the type I peptide was able to bind only to the reduced form of the COOH propeptide. Both rabbit polyclonal and murine monoclonal antibodies directed to the type III synthetic peptide bound the intact and the individual chains of type III procollagen as well as the intact and reduced forms of the type III COOH propeptide. The antibodies have been used to detect procollagen synthesis in two human osteosarcoma cell lines and the differential expression of procollagen in the culture medium of rat lung fibroblasts grown in the presence or absence of glucocorticoids.
- MEDLINE on STN **DUPLICATE 25** L37 ANSWER 48 OF 61 Dimethylnitrosamine (DMN)-induced liver fibrosis was used as an AB experimental model to study the relationship between serum concentrations of the N-terminal propeptide of type III procollagen [S-Pro(III)-N-P] and the N-terminal (S-7S) and C-terminal (S-NC1) domains of type IV collagen and hepatic concentrations of type III and IV collagen Increases in S-Pro(III)-N-P, and especially in the two type IV collagen-related antigens, were found to be early events in the formation of DMN-induced hepatic fibrosis. The mean concentration of S-Pro(III)-N-P was 120% of the control mean on day 7 of DMN treatment, 230% on day 14 and 250% on day 21. The corresponding values for S-7S were 260, 950 and 1100% and, for S-NC1, 310, 820 and 1000%. All these changes were very similar to those found in the hepatic concentrations of the respective mRNAs. These data support a previous suggestion that an enhanced production of basement-membrane (type IV) collagen is an early event in the development of the DMN-induced hepatic fibrosis. The results also indicate that S-7S and S-NC1 are very sensitive indicators of changes in type IV collagen metabolism. Data obtained in gel-filtration experiments for these three serum antigens were consistent with the suggestion that all three antigens are mainly derived from the synthesis of the respective collagens.
- ANSWER 52 OF 61 MEDLINE on STN DUPLICATE 28

  We evaluated the effects of a synthetic copy of a highly conserved portion (residues 225-246) of the COOH-propeptide of human pro-alpha 2(I) procollagen on collagen, fibronectin, and total protein synthesis by human fibroblasts. Incubation of COOH-propeptide 225-246 with fibroblasts resulted in a concentration-dependent inhibition of both type

I procollagen and fibronectin when compared with controls; a 50% inhibition of both fibronectin and type I collagen was observed at a concentration of 45 microM. Since the overall cellular protein synthesis was only minimally affected, COOH-propeptide appeared to specifically inhibit collagen and fibronectin synthesis. The peptide was nontoxic to cells and the inhibition was completely reversible upon removal of the peptide. We measured the steady-state levels of mRNAs coding for procollagen, fibronectin, and beta-actin by hybridization to specific recombinant cDNA probes; there was no significant change in the steady-state level of mRNAs of the three proteins. These results strongly suggest that the biosynthesis of procollagen and fibronectin in COOH-propeptide-treated cells is inhibited at a post-transcriptional level. These data establish a link between collagen and fibronectin synthesis and further define the important interaction of these molecules in the formation of the extracellular matrix.

- L37 ANSWER 59 OF 61 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 34
- The complete amino acid sequence of the carboxyl propeptide of AB chicken type II procollagen was determined by nucleotide sequencing of 3 recombinant plasmids harboring inserts. complementary to type II collagen mRNA. A recombinant plasmid containing sequences from the 3'-non-translated region of type II collagen mRNA was characterized. Since the nucleotide sequences did not correspond to regions of chicken type II procollagen for which protein sequence data exist, the physiologically cleaved type II carboxyl propeptide was purified from organ cultures of chick embryo sternal cartilages and its amino-terminal amino acid sequence was determined by automated Edman degradation. A comparison of the nucleotide-derived sequence with the sequence obtained by Edman degradation of the type II carboxyl propeptide provides definitive proof that the recombinant plasmids contain sequences specific for type II procollagen and allows for the elucidation of the cleavage site for procollagen C-protease within type II procollagen. The results of sequence analysis indicate that the type II carboxyl propeptide contains 246 amino acid residues. When the peptide is compared with the homologous region of pro  $\alpha 1(I)$  chains, the type II carboxyl propeptide appears to have an inserted amino acid residue in position 7 (counted from the C-protease cleavage site) and a deleted amino acid residue at position 101. The type II carboxyl propeptide is similar to that of pro  $\alpha 1(I)$  chains in that it contains 8 cysteinyl residues in the same positions, but it is different from the pro  $\alpha 1(I)$  peptide in that it contains 2 potential sites for N-linked oligosaccharide side chains while the pro  $\alpha 1(I)$ peptide contains only 1 such site.

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